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June 6, 2005

VIA ELECTRONIC AND U.S. MAIL

Ms. Cynthia Oshita
Office of Environmental Health Hazard Assessment
1001 I Street
P.O. Box 4010
Sacramento, CA 95812-4010

**Re: Comments of the Administration of Idaho Governor Dirk Kempthorne
Potential Regulatory Action Exempting from the Proposition 65 Warning
Requirements, Exposures from Chemicals That Form from Natural
Constituents in Food During Cooking or Heat Processing
Notice to Interested Parties Dated April 8, 2005 and
May 9, 2005 Workshop**

Dear Ms. Oshita:

On behalf of the Administration of Idaho Governor Dirk Kempthorne, we are hereby submitting comments of the Idaho Department of Agriculture, the Idaho Department of Commerce and Labor, the Idaho Department of Environmental Quality, and draft studies by Dr. Larry Branen (as well as his colleague Dr. Jerry Exon) of the University of Idaho addressing the matters set forth in the April 8, 2005 Notice to Interested Parties and discussed at the May 9, 2005 Workshop conducted by the Office of Environmental Health Hazard Assessment (OEHHA). Each of these parties personally appeared in Sacramento at the Workshop, and their May 9 statements are already part of the Workshop record and are incorporated herein by reference.

These comments are intended to provide additional perspective to the potential impact of Proposition 65 warnings for agricultural commodities grown in Idaho and sold in California. Governor Kempthorne and his Administration well understand issues surrounding reconciliation of legislation - in the case of Proposition 65, legislation

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enacted *by the People of California* - and the discharge of executive branch responsibilities.

The Kempthorne Administration does not question the wisdom of California's electorate in enacting Proposition 65, and nor does the Administration challenge the justification for the listing of acrylamide in the first instance. The Governor and his represented Idaho agencies in this matter strongly support an exemption framework that is both consistent with the wishes of Californians to be meaningfully informed about certain chemical exposures and the integrity of Proposition 65's original vision.

The statutory directive to California's State agency with lead responsibility for ensuring that Proposition 65 is appropriately implemented does not necessarily require OEHHA to consider the impacts of its regulatory decisions on Idaho.

However, the important decision to proceed (or not proceed) with this exemption should not be undertaken in a legal, policy, or science vacuum. The potential consequences for failing to provide the proposed exemption on Idaho's agricultural economy, *see* attached comments of Patrick A. Takasugi, Director of the Idaho State Department of Agriculture, and California's commercial marketplace for Idaho's signature commodity – potatoes – is important contextual information for well-informed decision making, *see* attached comments of Roger Madsen, Director, Idaho Department of Commerce and Labor. This exemption is also appropriate based on the current state of science, *see* attached draft studies of Drs. Branen and Exon, University of Idaho, and comments of Toni Hardesty, Director, Idaho Department of Environmental Quality.

The Workshop participants were specifically requested by OEHHA to set forth their views on the lawful basis under Proposition 65 for providing the exemption under discussion. The attached comments clearly express Idaho's support for this narrow exemption as a matter of California law. As will be discussed below, the proposed exemption does not require OEHHA to misapply or "waive" Proposition 65 in any fashion.

I. Introduction

Governor Kempthorne and his agency representatives who traveled to Sacramento to participate in the May 9 Workshop fully support development of the present

exemption framework. The Idaho agencies appreciate the opportunity to work with Dr. Denton and her staff at OEHHA to address some of the issues surrounding this approach.

The legal framework for this exemption rests comfortably within a judicial construction of Proposition 65 which concluded that the initiative was well able to accommodate a regulatory exemption similar in concept to that advanced by the Health and Welfare Agency in 1988 and proposed here. This approach is faithful to the framework of Proposition 65 as well as its interpretation by California courts.

II. Proposition 65's Legal Framework for the Exemption to Warning Requirements for Chemicals Formed From Cooking or Heat Processing

A. *Nicolle-Wagner v. Deukmejian*, 230 Cal. App. 3d 652, 281 Cal. Rptr. 494 (Cal.Ct.App. 1991)

The legal construct for the proposed exemption described in the April 8 Notice to Interested Parties has already been provided by one of Proposition 65's few reported California cases. In *Nicolle-Wagner v. Deukmejian*, 230 Cal. App. 3d 652, 281 Cal. Rptr. 494 (Cal.Ct.App. 1991), a citizen plaintiff challenged a newly-promulgated regulation which provided certain exemptions from an "exposure" under Proposition 65.¹

The plaintiff contended that Proposition 65 created *no categorical exception* for naturally occurring carcinogens or naturally occurring reproductive toxins, which, he claimed, were as threatening to health as man-made toxins. On appeal to the Second District Court of Appeal from a Superior Court decision upholding the regulation, the

1. The regulation reviewed in *Nicolle-Wagner* provided that "Human consumption of a food shall not constitute an 'exposure' for purposes of [California] Health and Safety Code section 25249.6 to a listed chemical in the food to the extent that the person responsible for the contact can show that the chemical is naturally occurring in the food." CAL. CODE REGS., tit. 22, § 12501(a) (1988). Under the regulation, a chemical is considered "naturally occurring" if "it is a natural constituent of a food, or if it is present in a food solely as a result of absorption or accumulation of the chemical which is naturally present in the environment in which the food is raised, grown, or obtained . . ." *Id.* § 12501(a) (1).

issue squarely before the Court was whether the regulation conflicted with or was not reasonably necessary to effectuate the statutory purpose of Proposition 65.

The Court of Appeal began its analysis by finding that Proposition 65 was silent on the subject of naturally occurring carcinogens and reproductive toxins and that the original intent of the initiative was to regulate toxic substances "which are *deliberately* added or put into the environment by human activity." *Nicolle-Wagner*, 230 Cal. App. 3d at 659 (emphasis added).

After reviewing the 1986 ballot arguments both supporting and opposing the passage of Proposition 65, the Court of Appeal was "persuaded, on balance, that the better view is that the electorate did not intend naturally occurring substances to be controlled by Proposition 65. Use of the terms such as 'knowingly and intentionally' and 'putting' imply that human conduct which results in toxins being *added* to the environment is the activity to be controlled." *Nicolle-Wagner*, 230 Cal. App. 3d at 660. Thus, the Court of Appeal concluded that Section 12501 was consistent with the governing statutes of Proposition 65.

Addressing whether the regulation reasonably effectuated the statutory purpose of Proposition 65, the Second District Court of Appeal pointed to an administrative record depicting that most food products contained at least trace amounts of carcinogens and reproductive toxins which were already listed of Proposition 65 chemicals. After taking note of the paucity of scientific data regarding the risk posed by such exposures, the Court of Appeal declared that:

We all presume, to some extent, that foods that have been eaten for thousands of years are healthful, despite the presence of small amounts of naturally occurring toxins.

...

The administrative record in this matter indicates that such evidence [of significant exposure risks] largely does not exist. Thus, grocers and others would be required, in order to avoid liability under these statutes, to post a warning label on most, if not all, food products.

...

Since one of the principal purposes of the statutes in question is to provide 'clear and reasonable warning' of exposure to carcinogens and

reproductive toxins, such warnings would be diluted to the point of meaninglessness if they were to be found on most or all food products.

Nicolle-Wagner, 230 Cal. App. 3d at 660-661.

According to the Court of Appeal, the exemption furthered Proposition 65 by safeguarding the effectiveness of warnings and in removing from regulatory scrutiny substances which pose only an insignificant risk of cancer or birth defects within the meaning of the statute. *Nicolle-Wagner*, 230 Cal.App.3d at 661. Importantly, the regulation was narrowly drawn and was not applicable to other products such as pharmaceuticals and cosmetics. *Id.*

B. An Exemption for Chemicals Formed by the Cooking or Heating of Natural Constituents is Not in Conflict with Proposition 65 and Furthers Its Statutory Purpose

Nicolle-Wagner held that a simple "exposure" of listed chemicals in food does not *de facto* trigger a warning under Proposition 65. Rather, California law requires some degree of conscious human activity which results in toxins being *added* to the environment to trigger the initiative's warning requirement. *See Nicolle-Wagner*, 230 Cal.App.3d at 660 (noting that "Proposition 65 expressly indicated that only 'man-made' substances would be regulated.")

Nicolle-Wagner also teaches that Proposition 65 requires warnings that must be "clear and reasonable," meaning that if such warnings are found on *most* or *all* food products sold in California, the result may be a public not well-served by the initiative. *Nicolle-Wagner*, 230 Cal.App.3d at 661. Stated differently, the statutory purpose of Proposition 65 is furthered by avoiding warnings on substances posing uncertain exposure risks. *Id.*

III. The Proposed Exemption is Consistent with Proposition 65 and Effectuates its Purpose

The proposed exemption to Proposition 65's warning requirements is consistent with the judicial construction of Proposition 65 in *Nicolle-Wagner*. First, any acrylamide formed by cooking is not "knowingly and intentionally" *added* into the human

environment by cooking or heating the natural constituents of the food. *Nicolle-Wagner*, 230 Cal.App.3d at 660.²

Second, one of Proposition 65's important interests, namely, safeguarding "the effectiveness of the warnings," *Nicolle-Wagner*, 230 Cal.App.3d at 661, is served by an exemption for exposures in food that result from an unintended byproduct of cooking. One alternative to the exemption proposed here is warning for *any* acrylamide exposure in food, which, due to the potential reach of such warnings, would have the real potential of creating "warning fatigue" in California. *Id.* (noting with approval the Final Statement of Reasons which declared that "unnecessary warnings ... could distract the public from other important warnings on consumer products.")

Finally, like the regulation upheld in *Nicolle-Wagner*, the proposed exemption will be purposefully calibrated to avoid overbreadth. Any exemption will only be triggered by exposures where food is cooked or heated and listed chemicals are created (not added) by a process causing a reaction of the food's *natural* constituencies.³ It will not be applicable to any other product than food. *See Id.* (noting that Section 12501 was narrowly tailored because "the regulation is ... applicable only to foodstuffs and not other products, such as pharmaceuticals and cosmetics.")

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2. At the May 9 Workshop, a representative from the California League for Environmental Enforcement Now (CLEEN) testified that the legal propriety of proposed exemption is "simply not a question of whether it[']s an unintended or an intended byproduct. The statute does not deal with that. It's the knowingly – knowingly exposure statute – standard." May 9, 2005 Transcript at 183:13-16. This argument ignores a key component of Health and Safety Code Section 25249.6, specifically, that culpability under Proposition 65 cannot occur unless a person "knowingly *and* intentionally" exposes the public. This fundamental provision of Proposition 65 cannot be written out of the law as a key regulatory element under Section 25249.6.
 3. The exemption proposed here is structurally distinct from a concept addressed in the Final Statement of Reasons justifying the adoption of Section 12501. There, it was asserted by commenters that "customary methods of food processing" be included in Section 12501(3)'s menu of excluded human activity ("sowing, planting, irrigation"), thus providing the basis by which an "exposure" could be exempted. Here, the exemption will be limited to exposures in specific foods (not across-the-board) whereby a listed chemical is formed through the cooking of natural constituencies. The Proposition 65 requirement that a listed chemical must be *deliberately added* is not offended by this exemption.

Ms. Cynthia Oshita
Office of Environmental Health Hazard Assessment
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IV. Conclusion

Idaho Governor Dirk Kempthorne and his Administration strongly support an exemption from California's Proposition 65 warning requirements for exposures to chemicals formed by the cooking or heating of natural constituencies in food.

The OEHHA has a sound basis to move forward with a proposed rulemaking as matter of law and public policy under Proposition 65 in addition to the current state of the science. We urge that an articulation of this exemption advance to a proposed rulemaking as soon as practicable.

Very truly yours,

A handwritten signature in black ink, appearing to read "L. Michael Bogert", with a stylized flourish at the end.

L. Michael Bogert

Attachments

cc: Mr. Pat Takasugi, Director
Idaho Department of Agriculture
Mr. Roger Madsen, Director
Idaho Department of Commerce and Labor
Ms. Toni Hardesty, Director
Idaho Department of Environmental Quality
Dr. Larry Branen
Dr. Jerry Exon
University of Idaho



STATE OF IDAHO

DEPARTMENT OF AGRICULTURE

DIRK KEMPTHORNE
Governor

PATRICK A. TAKASUGI
Director

June 6, 2005

VIA ELECTRONIC AND U.S. MAIL

Ms. Cynthia Oshita
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**Re: Comments of the Idaho State Department of Agriculture
Potential Regulatory Action Exempting from the Proposition 65 Warning
Requirements, Exposures from chemicals That Form from Natural Constituents in
Food During Cooking or Heat Processing
Notice to Interested Parties Dated April 8, 2005 and
May 9, 2005 Workshop**

Dear Ms. Oshita:

The following are my comments pertaining to the matters discussed at the May 9, 2005 Workshop conducted by the Office of Environmental Health Hazard Assessment (OEHHHA).

First, the proposed exemption is appropriate for OEHHHA's consideration because **acrylamide is naturally-occurring, and farmers do not add it to foodstuffs**. We respectfully reject the statements made during the Workshop implying that acrylamide is a manmade contaminant.

There is no dispute – through all investigative and research analysis – that acrylamide is naturally occurring when common cooking techniques are used, specifically roasting, toasting, baking, grilling, frying, and microwaving. These cooking techniques provide us with safe, palatable foods. Telling consumers to eat uncooked foods in an effort to avoid acrylamide would only aggravate the health risks a Proposition 65 warning seeks to avoid. Further, as a naturally-occurring substance, we question whether acrylamide falls under the scope of the voter-approved initiative.

Second, any effort to warn on acrylamide exposure would be sweeping, not limited to one food. The focus should not be on potatoes, though we understand that is the focus of the Proposition 65 case in front of OEHHHA. We believe that our comments should be sufficiently worded to reflect the fact that multiple foods would be subject to acrylamide labeling under Proposition 65, if it is agreed that potatoes should be labeled. Thus, we would note that more than 30 foods

would fall under acrylamide labeling if potatoes themselves are labeled. Warnings would be included on items that include coffee, bread and cereals.

Finally, we understand that Proposition 65 is a right-to-know law, but what exactly are consumers being told if a food is labeled as containing acrylamide? The answer is that as a matter of furthering Proposition 65's statutory purpose, a warning about acrylamide exposure will not inform consumers much, if anything, about potential exposure.

Acrylamide is considered a "*possible* carcinogen." Beyond that, little is known about its health effects. The World Health Organization notes, "the theoretical models to predict whether cancer would develop in humans as a result of ingesting acrylamide in food are not reliable enough to develop firm conclusions about risk." The U.S. Food and Drug Administration notes it "is not able to make a determination regarding the public health impact of acrylamide from the very low levels found in foods." The agency is still studying potential health effects. The UK's Food Standards Agency says people shouldn't change their diets because of the recent discovery of acrylamide. Sweden's National Food Administration reports that studies in man "have not shown a correlation between exposure to acrylamide and increased cancer rate." Food Standards Australia New Zealand notes in its comments to consumers that Swedish researchers have found "a lack of an excess risk, or any convincing trend of cancer of the bowel, bladder or kidney in high consumers of fourteen different food items with a high or moderate range of acrylamide content." The European Union notes "the possible risk to public health is unclear."

No one denies the existence of acrylamide in food, but no one can say what it means that acrylamide exists. Until we understand more, warning that acrylamide exists tells the consumer nothing. No massaging of the inconclusive data can provide an accurate statement. Further, food agencies world wide are advising people to eat a balanced diet, choosing a variety of foods that are low in trans fat and saturated fat, and rich in high-fiber grains, fruits, and vegetables. Acrylamide warnings may result in an adverse effect on positive, healthful food consumption.

Sincerely,

Patrick A. Takasugi
Director
Idaho State Department of Agriculture

pc: Governor Dirk Kempthorne
Mr. A.G. Kawamura, Secretary, California Department of Food and Agriculture

Dr. Robert G. White, President
 1984-1985

The importance of open economic exchange among the states cannot be understated, especially as the United States demands free and fair trade among the nations of the world.

But while we in Idaho are as concerned as anyone about the safety of the food and other commodities our farmers and businesses ship not only to other states but across the globe, we believe there is not the scientific basis to justify imposition of a consumer warning on French fries at this time.

This is a naturally occurring substance in the processing of high-carbohydrate foods – foods that Americans and people all over the world have been eating for generations, seemingly without adverse health effects. Over the past two centuries, French fries have become a staple for many – the comfort food of choice for students, as Doctor Branen suggested.

There is no forgetting the impact on the apple market from the 1989 report falsely linking the chemical Alar to cancer. That unfounded health scare cost that industry hundreds of millions of dollars and prompted tens of thousands of consumers to shun a healthy food that has significant dietary benefit.

As that research goes on, food producers have already begun looking for improved processing and preparation techniques that can reduce acrylamide levels in food, and nutritionists continue to emphasize the importance of a balanced diet and healthy eating habits.

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A third of the potatoes grown in the United States come from Idaho. They contribute markedly to our economy, especially in the more rural south-central and eastern parts of the state.

And they are part of the billions of dollars in goods Idaho ships to California each year. In 2002, the Bureau of Transportation Statistics reported Idaho moved \$4.6 billion in products to California, and California businesses shipped \$2.2 billion in goods to Idaho. The Los Angeles and Long Beach ports alone handle about \$30 million a year in Idaho products for overseas buyers and foreign goods destined for Idaho.

Admittedly, that is only a fraction of the interstate trade California conducts each year. But it can easily be disrupted by the fallout from a consumer warning built on an insufficient scientific foundation.

The flow of goods not just with Idaho but with all the other states, especially in the West, provides the backbone for a strong regional and national economy. We believe that trade is critical to the entrepreneurs depending on it – both in California and the other states.

Washington and Oregon, for example, produce a fifth of the nation's potatoes, and growers there have the same concerns as ours, and those two states had over \$50 billion in total trade with California in 2002.

It is the reason maintaining a positive business climate is important to everyone.

As regulators, it is imperative you find a reasoned approach to handling a food safety and nutritional issue that scientists have only begun analyzing and remain uncertain about its health effects, if there are any.

Such an approach will maintain the positive economic climate that encourages unfettered interstate commerce.

THE CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY

OFFICE OF ENVIRONMENTAL HEALTH HAZARD ASSESSMENT

WORKSHOP ON POTENTIAL REGULATORY ACTION EXEMPTING FROM THE PROPOSITION 65 WARNING
REQUIREMENTS, EXPOSURES FROM CHEMICALS THAT FORM FROM NATURAL CONSTITUENTS IN FOOD
DURING COOKING OR HEAT PROCESSING

Scientific Uncertainties:

The Need for a Cooking and Heat Processing Exemption

Submitted by:

***Toni Hardesty, Director
State of Idaho
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June 6, 2005

The Idaho Department of Environmental Quality (DEQ) is headquartered in Boise, Idaho. DEQ is the state-level entity that is charged with the task of protecting human health and preserving the quality of Idaho's environment.

Idaho supports the Office of Environmental Health Hazard Assessment (OEHHA) on moving forward with the exemption rulemaking process as a sincere effort to come up with a fair and practical solution that is in the best interest of Californians and Idahoans alike ... a solution that is based on sound scientific information and fair and practical public policy practices.

Public Policy vs. Scientific Uncertainties

There are really two distinct discussions that need to take place regarding dietary exposure to Acrylamide. The first centers around science and the second centers around the broader public policy issues such as exposure risks vs. nutritional value and exposure risks vs. economic impacts. Our comments address the public policy portion of this discussion, but cannot do so without first touching on the science. The public policy discussion hinges (and rightly so) on the scientific information and our understanding of the risks associated with exposure to dietary levels of Acrylamide.

As you are well aware, the scientific information available, regarding the health effects associated with exposure to dietary levels of Acrylamide, is somewhat incomplete. There have been no conclusive findings that link low level, dietary exposure to Acrylamide to increased risk of neurological, reproductive, or cancer effects. As a matter of fact, the consensus among the scientific community seems to be that further research needs to be conducted and that a continued dialogue needs to take place to truly understand the risk associated with exposure to dietary Acrylamide versus the benefits received from consuming the foods that contain it (Branen). There have been studies that identified enough concern over dietary exposure to Acrylamide to warrant further discussion, however, the overall body of scientific knowledge is not complete so more research is needed.

As regulators, responsible for protecting public health, we have a responsibility to base our decisions on sound scientific information. I would argue that the body of scientific knowledge regarding exposure to dietary Acrylamide is not yet at a point where it can properly inform the public policy discussion. Moving forward on other regulatory proposals could result in unwarranted consumer fears and unintended consequences. It's a well known fact that incidences of cancer are on the rise. However, the underlying cause for this increase is still very much unknown.

Given the uncertainty in the science, the logical choice should be to exempt exposures for chemicals that form from natural constituents in food during cooking or heat processing.

This exemption should be in place until such time that there is a better understanding of the risks associated with low-level, dietary exposure versus the benefits associated with consumption of the foods, and regulators can make an informed decision on the steps (if any) that should be taken to warn consumers.

Acrylamide Content of Food Products

May, 2005

**Larry Branen
Professor of Food Science
Center for Advanced Microelectronic and Biomolecular Research
and
College of Agricultural and Life Sciences
University of Idaho**

Acrylamide Content of Food Products

Executive Summary

Acrylamide is a natural byproduct in certain carbohydrate-rich foods that forms when these foods are fried, baked, or roasted at high temperatures. Through these cooking processes the safety, digestibility and acceptability of foods is significantly increased. Unfortunately the Maillard reaction, that is responsible for much of the flavor and color development in cooked food products, can also result in the formation of acrylamide under certain conditions. The Maillard reaction results from a complex set of reactions between reducing sugars and amino acids. In food products containing sufficient quantities of the free amino acid, asparagine, and reducing sugars, the Maillard reaction can result in the formation of acrylamide. Since the first identification of acrylamide in food products in 2002, numerous studies have been completed on the mechanism of formation of acrylamide, the methodology used to determine acrylamide, and the reported content of acrylamide in food products and in the diet. One of the major limitations of studying the formation and level of acrylamide in food products is the lack of adequate methodology for detection of acrylamide at the low and varied levels in food products. Although there is no required or approved method by the AOAC International for determining acrylamide content of foods, the use of liquid chromatography/tandem mass spectrometry (LC-MS/MS) has become the standard method and is the recommended method of FDA.

A number of studies have been completed worldwide on acrylamide content of food products; however, the reports include only a few selected foods and do not represent a total food review. Due to the variation in analytical techniques and sampling methods there is also a significant variation in the levels reported. Both the level of acrylamide precursors in the raw products as well as the processing conditions can significantly influence the level of acrylamide in the final product. Carbohydrate-rich products containing asparagine and processed at high temperatures contain the highest levels of acrylamide. Commonly consumed foods such as potatoes, almonds, cocoa, wheat grain, olives and rice all show relatively high amounts of asparagine and when processed through heating show relatively high amounts of acrylamide. All of these foods are listed on FDA's list of the top twenty foods contributing to acrylamide consumption in the US. The FDA has estimated exposure from acrylamide in foods to be 0.4 ug/kg-body weight-day in the US while the worldwide estimated exposure is 0.3 to 2.0 ug/kg-body weight-day.

Research on the content of acrylamide in foods and the significance of these levels should be continued in the future. Methodology for analysis of acrylamide in foods needs to be standardized and applied to ongoing surveys of food products and methods should also be explored for the reduction of acrylamide formation in food products. Like so many other choices in food consumption, the reduction in acrylamide in foods may come at the cost of the desired flavor, color, safety and digestibility of food products. Caution must also be taken in the potential labeling of foods containing acrylamide. Unwarranted consumer fears could lead to avoidance of foods that contribute significantly to the nutritional value of the American diet. Informed scientists, food processors, consumers and legal authorities must continue to meet on a worldwide basis to better understand the safety of foods containing acrylamide and to develop ways to balance the potential risks of the acrylamide in the foods versus the benefits of consuming these foods.

Background

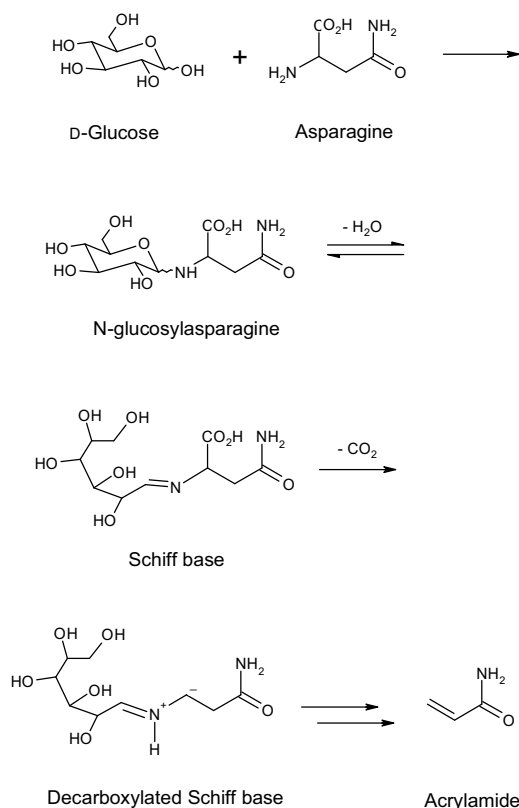
Acrylamide was first reported in food in 2002 by Swedish scientists (Tareke, et al., 2002) and since that time there have been numerous studies on the formation and level of acrylamide in food products. It is now well-recognized that acrylamide is a natural byproduct in certain carbohydrate-rich foods that forms when these foods are fried, baked, or roasted at high temperatures. Although high doses of acrylamide have been reported to cause cancer and reproductive problems in laboratory animals, the true public health significance to humans of the known levels in food products is not well understood. Nevertheless, several consumer groups have expressed concern about the levels of acrylamide in foods and Proposition 65 in California could lead to labeling of foods with relatively high levels of acrylamide (Joy, 2003; Duxbury, 2004). The US Food and Drug Administration as well as other regulatory agencies worldwide have developed action plans to guide activities on acrylamide detection in food products. There has been extensive research in the areas of methodology, toxicology, and acrylamide formation and data have been released periodically on acrylamide levels in food. The focus of this review is on the mechanism of formation of acrylamide in food products, the methodology used to determine acrylamide, and the reported content of acrylamide in food products and in the diet.

Mechanism of Formation of Acrylamide in Food Products

It has been recognized since the beginning of time that the cooking of foods not only serves to enhance the safety and digestibility of foods, but also serves to develop the desired flavor, color and texture of numerous cooked foods. Nonenzymatic browning, the Maillard reaction, is responsible for much of the flavor and color development in cooked and browned food products. The mechanism for this reaction has been well-characterized and is known to result from the reaction of reducing sugars (carbonyl moiety) and the amino group of amino acids and other amine compounds (deMann, 1999). This complex set of reactions is known to occur at temperatures above 120° C and to result in the formation of numerous flavor and color compounds. Much work has been done to optimize the development of the desirable flavor and color compounds produced in food products and in some cases to limit the overall reaction through reducing the level of the reactants (reducing sugars and amino acids and proteins), control of temperature and moisture, and the addition of food additives.

Unfortunately it is this same reaction that results in the formation of acrylamide in food products. Although according to Stadler et al. (2004), several minor pathways may contribute to the formation of acrylamide, the major pathway under low-moisture conditions and elevated temperatures is via the reaction of asparagine (an amino acid) with reducing sugars. It is now well-accepted that in food products containing sufficient quantities of free (non bound) asparagine, the reaction of the α -amino group of asparagine with reducing sugars can result in the formation of acrylamide (Zyzak et al., 2003; Mottram et al., 2002). It is important to recognize that the reaction requires the presence of both substrates and through a complex set of reactions results in some of the same flavor and color compounds derived from this reaction from other amino acids. Although acrylamide is not the favored product with a reaction efficiency of about 0.1% (Becalski et al., 2003; Stadler et al., 2004), it is able to accumulate to detectable levels in food products subjected to prolonged heating. Research in both buffered and model food systems (Zyzak et al., 2003; Mottram et al., 2002) have confirmed that the reaction most likely occurs via the glycosyl-asparagine derivative (Schiff base), which then undergoes

decarboxylative deamination to form acrylamide, whose atoms are derived solely from the asparagine molecule. These reactions are summarized as follows:



Acrylamide formation requires a minimum temperature of 120° C (thus, does not occur in boiled foods), and is kinetically favored with increasing temperatures approaching 175-200° C (Robert et al., 2004; Mottram, 2002). With extended heating above 175° C, acrylamide levels may actually decrease via thermal elimination/degradation reactions. Food levels of acrylamide are also impacted by pH. Acrylamide formation is favored as the pH is increased over the range of 4 to 8 and the maximal level appears to occur at a pH of 8 (Ryberg et al., 2003). The reduced acrylamide formation in the acid range is thought to be due in part to protonation of the asparagine α -amino group, effectively reducing the reactivity of the amino acid. Further, in an acidified food medium, acrylamide also appears to be subject to increased rates of thermal degradation that may also contribute to the pH phenomenon. In model systems of glucose and asparagine, water activity does not appear to have a direct role in acrylamide formation as approximately equal amounts of acrylamide are formed in both dry and high moisture heated conditions. However, in practical food applications, acrylamide levels have been shown to increase rapidly in the latter stages of prolonged heating processes, presumably as the water at food surfaces is driven off to allow surface temperatures to attain the needed levels for acrylamide formation. Products with high amounts of surface area (i.e. potato chips) are among those high-temperature processed foods that exhibit the highest acrylamide levels. Thus, the exposed surface area of a food can be an additional factor in acrylamide formation provided that reaction substrates and processing temperatures are sufficient to drive acrylamide formation.

Methodology for Detection of Acrylamide in Foods

One of the major limitations of studying the formation and level of acrylamide in food products is the lack of adequate methodology for detection of acrylamide at the low and varied levels in food products. Due to the low quantities of acrylamide in food products, methods must include both effective extraction and enrichment procedures as well as sensitive methods for detection. Several recent reviews provide excellent summaries of the current methodology (Dybing et al., 2005; Claeys et al., 2005; Stadler and Scholz, 2004; Zhang et al., 2005). Zhang et al. (2005) concludes in their review that despite a significant improvement of the procedures in recent years, there still remains the need to develop stable, reliable and robust methods for difficult matrices. They also point out the need for statistically sound sampling techniques. Currently there is no required or approved method by the AOAC International for determining acrylamide content of foods. Both gas chromatography and liquid chromatography methods have been developed; however, the use of liquid chromatography/tandem mass spectrometry (LC-MS/MS) has become the standard method for most researchers and is the recommended method of FDA (Alan et al., 2002; Duxbury, 2004). The initial use of LC-MS/MS for acrylamide detection was developed and published by Rosen and Hellenas (2002) and was the method used when the initial report of acrylamide in food was made by these researchers in 2002. Gas Chromatographic methods have been developed by several researchers with some success and they offer a less expensive method than the LC-MS/MS method (Zhang et al., 2005). Alan et al. (2002) found good agreement between a GC-MS and LC-MS/MS method for analyses of acrylamide in food products. Yasuhara et al. (2003) also reported the use of a GC with a NPD detector and reported that for his model studies, the GC/NPD method was comparable to an LC/MS method. Overall it appears that the LC-MS/MS provides the most accurate and precise measurements of acrylamide in foods and according to Henry B. Chin of the National Food Processors Association is the first choice for analytical methodology for acrylamide (Duxbury, 2004). Wenzl et al. (2004) carried out an extensive evaluation of results from 62 laboratories applying different measurement techniques and a broad spectrum of sample extraction and preparation procedures applied to crispbread and cookies. They concluded that there was a significant influence of the analysis technique and that there appeared to be a concentration-dependant and/or matrix dependant influence on the results from analysis. The quality of the results appeared to decrease for samples at or near the limit of quantification (30-50 ug/kg). The extraction procedures are also important in food analysis and thus the digestion of the product versus a water extraction can make a significant difference in the results obtained. All of this must be taken into account in evaluating the information reported from several food studies. The coordinated effort by NEPA and FDA should help in obtaining reliable information with the development of a standardized method for acrylamide analysis of food products. A summary of current research on methodology for acrylamide can be found at the European Union website on acrylamide (http://europa.eu.int/comm/food/food/chemicalsafety/contaminants/acryl_database_en.htm).

Reported Levels of Acrylamide in Foods

Since the initial release of data on the presence of acrylamide in food products, a number of studies have been completed worldwide. There are now a number of websites that provide public information on the level of acrylamide in various food products. Unfortunately due to the variation in analytical techniques and sampling methods there is a significant variation in the

levels reported for various foods. As well, based on the mechanism of formation of acrylamide both the level of acrylamide precursors in the raw products as well as the processing characteristics (time and temperature) can significantly influence the level of acrylamide in the final product. Also the method of reporting and data analysis varies with each report. In some cases averages or means are reported while in others only the ranges are provided with limited statistical evaluation of the data. The most comprehensive report on US products has been reported by the FDA at the Center for Food Safety and Applied Nutrition (CFSAN) on their website (<http://www.cfsan.fda.gov/~dms/acrydino.html>). A summary of that data is given in Table 1. Although other studies have shown other content levels, the range of levels reported by the FDA is very close to that of others. Freidman (2003) has summarized much of the content information on a worldwide basis in his excellent review on acrylamide and the reader is referred to that paper for additional information. For more specific detail on individual foods, the reader is also referred to the individual studies that have been published on the above website as well as in several recent reviews (Dybing et al., 2005; Stadler and Scholz, 2004; Zhang et al., 2005 Office of Environmental Health Hazard Assessment, 2005).

The data confirm the tremendous variation in acrylamide levels as well as the breadth of food products that contain acrylamide (Table 1). It is important to note that there is a great deal of variation in the reported levels within these products as well as within each product category. Also a caution should be stated that the focus of the studies has been on selected foods and the studies do not represent all of the foods that could potentially contain acrylamide. It is expected that additional food items will be added each year by the FDA. As an example, canned black olives, Postum and prune juice were not included in the survey by the FDA until 2004 and all of these foods were found to contain significantly high quantities of acrylamide (DiNovi and Howard, 2004). As one would expect from the chemical mechanism for formation of acrylamide, carbohydrate-rich products processed at high temperatures contain the highest levels of acrylamide. In addition there appears to be a direct correlation between the level of free asparagine in these food products and the potential for acrylamide formation. Friedman (2003) has summarized current information on the level of asparagine in various food products. Potatoes, almonds, cocoa, wheat grain, and rice all show relatively high amounts of asparagine and when processed through heating also show relatively high amounts of acrylamide. In comparison, several vegetables including asparagus, broccoli, green beans, and cauliflower also show high quantities of asparagine, but since these are normally not prepared or processed at the high temperatures, there has not been a high content of acrylamide reported for these foods after preparation.

Heating and processing techniques have a direct impact on the levels of acrylamide. It is evident from the data in Table 1, that cooking and/or heating of products such as potatoes, bread, cocoa and coffee significantly increase the level of acrylamide. It should be noted that much of this cooking and heating occurs in the home rather than in processing or restaurant facilities. This is something that cannot be monitored but could contribute significantly to the overall content of acrylamide in foods.

Acrylamide in Potatoes. The relatively high content of acrylamide in potato products is the result of the higher level of acrylamide precursors and the processing temperatures used for producing these fried products. As indicated above, acrylamide is derived primarily from the

reaction between reducing sugars and free amino acids, both of which are in high quantities in potatoes (Friedman, 2003). Asparagine is the free amino acid present in the highest amount in potatoes and the quantity of this amino acid has been shown to vary among cultivars (Amrein et al., 2003; Becalaski, et al., 2004). Studies in both Canada and Europe have also shown a correlation between the level of asparagine and the level of acrylamide in the processed potato products produced from these materials (Amrein et al., 2003; Becalaski, et al., 2004). However, these and other studies have concluded that the level of reducing sugars in the potato cultivars was the primary determining factor in acrylamide formation (Biedermann-Brem et al., 2003; Williams, 2005). According to Amrein et al. (2003) the level of reducing sugars varies in the selected potato cultivars to a greater extent than the level of asparagine and thus the level of reducing sugars is the determining factor in acrylamide formation. Becalski et al. (2004) suggested that the current practice of selecting material low in reducing sugars through selection of varieties and development of growing and storage profiles may be the best way of preventing acrylamide formation in finished potato products. Amrein et al. (2004) and Beidermann et al. (2002), however, concluded that neither the farming system nor the extent of nitrogen fertilization influenced the level of the reducing sugars and asparagine and thus the potential of acrylamide formation. Storage conditions, however, are well known to influence the level of reducing sugars and Chuda et al. (2003) concluded that the level of acrylamide in potato chips was ten times higher in potatoes stored at 2° C versus 20° C. Olsson et al. (2004) also showed that asparagine levels did not change during storage of potatoes. Amrein et al (2003) recommended that future efforts should focus on cultivar selection to reduce acrylamide production as long as storage temperatures below 8-10° C are avoided.

Although the composition of the raw material may be the most important factor, processing conditions also play a major role in the formation of acrylamide in the finished products. The temperature of processing is probably the most important factor with acrylamide formation requiring a minimum temperature of 120° C and increasing as temperatures increase toward 200° C. Thus the par or pre-frying process which is carried out at 140° C results in significantly lower acrylamide levels than the final frying process with temperatures approaching 180° C (Grob et al., 2003). Minimizing the temperature can be advantageous; however, it must be balanced against the loss of the desirable flavor, texture and color resulting from the heating process (Williams, 2005). Taubert et al. (2004) reported that surface area and processing time are important determinants of acrylamide generation during frying of potato products and that high surface browning does not generally indicate high acrylamide content. Other work has been done to minimize acrylamide formation through the use of blanching to remove the acrylamide precursors and the use of various additives to limit the browning reaction and thus the acrylamide formation (Tricoit et al., 2004; Lindsay and Jang, 2004).

Acrylamide in Cereal Products. Most cereals contain a significant quantity of asparagine and thus under the right conditions of temperature and in the presence of reducing sugars, acrylamide is formed in cereal products. As would be expected, the process of toasting results in higher quantities of acrylamide in bread products (See Table 1.) Ahn et al. (2002) showed a direct correlation between toasting time and acrylamide concentration in both white and brown breads. Surdyk et al. (2004) studied the impact of asparagine, fructose and baking conditions on acrylamide content in yeast-leavened wheat bread and determined that 99% of the acrylamide was formed in the crust and that while asparagine increased acrylamide formation, added fructose

did not influence content. Temperatures above 200° C and time influenced content and there was a direct correlation between color and acrylamide content, although added asparagine did not influence color indicating that other amino acids were responsible for color development. Elmore et al. (2005) reported that in cakes made from wheat and rye, as well as potato flakes, acrylamide formation did not occur to a large degree until the moisture contents of the cakes fell below 5%.

Estimated Consumption of Acrylamide from Food Products

As a part of the FDA Action Plan, in 2004 the FDA published data on the exposure estimate for acrylamide based on the residue data published on the FDA website and reported here in Table 1 and the total diet study done in 2004 (DiNovi and Howard, 2004). The current estimate of 0.4 ug/kg-body weight-day is identical to the first FDA model estimate in 2003 and falls within the estimated range of 0.3 to 0.8 ug/kg-bw-d done in 2002 by the FAO/WHO and the more recent estimate of 0.3 to 2.0 ug/kg-bw-d reported by FAO/WHO in their report at the February 2005 meeting (Joint FAO/WHO, 2005). Konings et al. (2003) had similar estimates for the Dutch population with an estimate of 0.48 ug/kg-bw-d, however, Boon et al. (2005) has recently reported a median intake of 0.5 ug/kg-bw-d for the Dutch population with a 1.1 ug/kg-bw-d for children.

The foods that contribute the most to the acrylamide exposure vary significantly based on the eating habits as well as the methods of preparation and processing of the foods in individual countries (Dybing, et al., 2005). Based on the residue data and the portion sizes of various foods, in the US, the FDA listed the following foods as the top eight foods in acrylamide per portion: breakfast cereal, brewed coffee, Postum, French Fries (regular fries and oven baked), potato chips, canned black olives, and prune juice. The FDA has also listed the top twenty foods contributing the highest to the mean daily intake of acrylamide in the diet with these same foods leading the list. According to Dybing et al. (2005) in countries where potatoes are consumed at relatively high amounts such as the US and the Netherlands, French fries and chips contribute the greatest to the total acrylamide intake. However, the contribution of bread and coffee is much higher in European countries. Dybing et al. (2005) also points out that information is lacking on the contribution of home cooked foods to total consumption and reports that home cooking could make a 50% contribution to overall acrylamide intake.

Even if the acrylamide in some of the high content level foods is reduced by new processing methods or these foods are eliminated or reduced in the diet, it is likely that only limited reductions will occur in total acrylamide exposure. Several researchers have used exposure assessments to model the effects of such mitigation strategies. Boon et al. (2005) modeled acrylamide reductions of 35 % in French fries and 60 % in gingerbread and found reductions in total acrylamide exposure for Dutch consumers of 13 % and 4 %, respectively.

The significance of the estimated consumption is not clear. The large variability in the levels reported for food products and the limited data available adds some uncertainty to the estimated consumption, but it is quite likely close to the this range. Dybing et al. (2005) has concluded that the exposure estimates are uncertain due to the underlying data as well as assumptions used in the exposure estimate methods. It should also be pointed out that it is not clear if the acrylamide in these foods is actually absorbed following consumption (Schabacker et al., 2004). The

estimated 0.4 ug/kg-bw-d would result in a daily exposure exceeding the no significant risk level (NSRL) established by the Office of Environmental Health Hazard Assessment (OEHHA) for Proposition 65 in California (<http://www.oehha.org/prop65.html>). The original NSRL was set at 0.2 ug per day; however, the OEHHA has proposed increasing this to 1 ug per day (Office of Environmental Health Hazard Assessment, 2005). It is likely that the levels of acrylamide exposure from foods have been in these same ranges since we started cooking foods. Although the processed foods are now more prevalent, the same processes used to produce the desired flavor and color of foods likely led to the development of significant quantities of acrylamide in home cooked foods for centuries.

Conclusions

Research on the content of acrylamide in foods and the significance of these levels should be continued in the future. Methodology for analysis of acrylamide in foods needs to be standardized and applied to ongoing surveys of food products. As is currently being done, methods should also be explored for the reduction of acrylamide formation in food products (Friedman, 2003; European Union, 2005; FDA, 2005). Most recommendations for reducing acrylamide are based on developing ways to reduce the acrylamide precursors (free asparagine and reducing sugars) and/or better control of the processing conditions that favor acrylamide formation (time, temperature, pH, water activity, surface area). Research is under way in all of these areas and should lead to some guidelines for processors and consumers in finding ways to limit the level of acrylamide in foods.

Like so many other choices in food consumption, the reduction in acrylamide in foods may come at the cost of the desired flavor and color of food products. A primary example is related to the breakfast cereals and nuts such as almonds that are listed near the top of the list for total acrylamide. These foods are known to play a major role in improving the nutrition for many children and adults in the U.S. and the flavor and color of these products that results from the heating process plays a major role in increasing the acceptability and safety of these products. Unfortunately the same natural nonenzymatic browning process that leads to the formation of the desired color and flavor can also lead to acrylamide formation. If warnings were to be required for consumers on any food containing acrylamide, it would impact more than 40% of the foods in the diet (Joy, 2003). Regulatory agencies must address the questions: Does the risk of acrylamide exposure from these foods outweigh the consumer's desire for a product that has desirable flavor and color? Does the food value derived from these products outweigh the potential risk from acrylamide exposure at the estimated levels in these products? Informed scientists, food processors, consumers and legal authorities must continue to meet on a worldwide basis to address concerns on an ongoing basis and develop ways to balance the risks of the acrylamide in the foods versus the benefits of consuming these foods.

Table 1. Reported Acrylamide Concentrations in food products.

Food Category	Food	Acrylamide Concentration* (µg/kg = ppb)
Beverages	Coffee (ground , not brewed)	37-374
	Coffee (instant, not brewed)	172-539
	Brewed Coffee	3-13
	Postum (Powdered)	3747-5399
	Postum (Brewed)	93
Breads and bakery products	Bagels(toasted and non-toasted)	12-343
	Breads (not toasted)	<10-130
	Breads (toasted)	59-364
	Pies/pastries	<10-74
Cereals	Breakfast cereals	11-1057
Chocolate Products	Cocoa	ND-909
	Chocolate mix	ND-45
Cookies and Crackers	Crackers, wheat thins	17-620
	Cookies	36-432
Dried foods	Noodles, soup mixes	<10-1184
Fruits and Vegetables	Canned fruits and vegetables	ND-83
	Canned Black Olives	123-1925
	Fresh fruits and vegetables	ND-<10
	French Fries (Before cooking)	20-218
	French Fries (After cooking)	117-1325
	Prune Juice	53-267
Gravies and Seasonings	Gravies and Seasonings	38-151
Nuts and nut butters	Almonds	236-457
	Peanut butter	64-125
Protein Foods	Chicken pieces	ND-35
	Meat and poultry	ND-30
Snack Foods	Potato Chips	117-2762
	Corn chips, popcorn, pretzels	12-990

Data abstracted from FDA data at the Center for Food Safety and Applied Nutrition (CFSAN) website (<http://www.cfsan.fda.gov/~dms/acrydino.html>). ND = not detected

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A REVIEW OF THE TOXICOLGY OF ACRYLAMIDE

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Abstract

Acrylamide (ACR) is a chemical used in many industries around the world and more recently has been found to form naturally in foods cooked at high temperatures. It has been shown to be a neurotoxicant, reproductive toxicant, and carcinogen in animal species. Only the neurotoxic effects have been observed in humans and only at high levels of exposure in occupational settings. The mechanism for the neurotoxic effects of ACR may be basic to the other toxic effects seen in animals. This mechanism involves interference with the kinesin-related motor proteins in nerve cells and eventual cell death. Neurotoxicity and resulting behavioral changes can affect reproductive performance of ACR-exposed laboratory animals with resulting decreased reproductive performance. Also, the kinesin motor proteins are important in sperm motility which could alter reproduction parameters. Effects on kinesin proteins could also explain some of the genotoxic effects on ACR. These proteins form the spindle fibers in the nucleus that function in the separation of chromosomes during cell division. This could explain the clastogenic effects of the chemical noted in a number of tests for genotoxicity and assays for germ cell damage. Other mechanisms of ACR toxicity are likely related to an affinity for sulfhydryl groups on proteins. Binding of the sulfhydryl groups could inactive proteins/enzymes involved in DNA repair and other critical cell functions. Direct interaction with DNA may not be a major mechanism of cancer induction in animals. The DNA adducts that form do not correlate with tumor sites and ACR is mostly negative in gene mutation assays except at high doses which may not be achievable in the diet. All epidemiologic studies fail to show any increased risk of cancer from either high level occupational exposure or the low levels found in the diet. In fact, two of the dietary studies show a decrease in cancer of the large bowel. A number of risk assessment studies have been performed to estimate increased cancer risk. The results of these studies are highly variable depending on the model, assumptions are made beyond the database and the values obtained are purely hypothetical. Regulatory agencies in several countries do not endorse the use of risk assessment models in estimating human cancer risk. There is universal consensus among international food safety groups in all countries that have examined the issue of ACR in the diet that not enough information is available at this time to make informed decisions on which to base any regulatory action. Too little is known about levels of this chemical in different foods and the potential risk from dietary exposure. Avoidance of foods containing ACR would result in worse health issues from an unbalanced diet or pathogens from under cooked foods. There is consensus that low levels of ACR in the diet are not a concern for neurotoxicity or reproductive toxicity and any relationship to cancer risk is strictly hypothetical.

Background

Recent studies show that many commonly consumed fried and baked foods have naturally occurring levels of acrylamide (ACR) (Tareke, Rydberg, *et al.*, 2000, 2002).

Several events led to this discovery. Workers building a railroad tunnel near the Bjare peninsula in southwest Sweden began to develop signs of impaired nerve function. This was eventually traced to exposure to a sealant called Rhoca-Gel that was used to water proof cracks in the tunnel wall. This sealant contained acrylamide (ACR) which had previously been shown to be a neurotoxicant in other occupational settings and animal studies (Spencer and Schaumburg, 1974). Subsequent studies on the tunnel workers were conducted to measure hemoglobin (Hb) adducts in the blood which are a biomarkers of ACR exposure (Hagmar, Tornqvist, *et al.*, 2001). It was discovered that the controls from this group also had equally high levels of the Hb adducts. This resulted in a search for the source of ACR exposure in the control subjects. Since it was known that ACR was formed from heating biological materials (i.e. tobacco), a dietary source was suspected. This eventually led to a study in rats fed fried food (Tareke, Rydberg, *et al.*, 2000). The rats developed the Hb adducts characteristic of ACR exposure. This prompted a more extensive study of ACR in different food products which was published by the Swedish government in 2002 (Tareke, Rydberg, *et al.*, 2002). Their study showed that starch-based foods that were fried or baked at high temperatures contained residues of ACR. Additional studies were done by various other countries (United States, United Kingdom, Canada, Norway, Australia) and international organizations (Food and Agriculture Organization-FAO/World Health Organization-WHO) that confirmed the Swedish results. These findings caused concern among food safety and regulatory agencies around the world because ACR had already been shown to be toxic to the nervous system in animals and humans and was a reproductive toxicant and carcinogen in animals. In fact, ACR was classified as “probable human carcinogen” by the International Agency for Cancer Research (IARC 1994). The results prompted several meetings at the international level which brought experts together to discuss the relevance of these findings. The statements issued from these meetings from the US Federal Food and Drug Administration, the UK Foods Standard Agency, Health Canada, Swedish National Food Administration and the United Nations Food and Agriculture Organization and the World Health Organization were almost universal. The conclusions were that this was a matter of concern for food safety but there was a lack of evidence of any effects of ACR exposure via dietary sources in humans. None of the agencies or groups recommended any changes in our food choices. In fact, because of the wide range of foods that may have ACR residues, any attempt to try exclude these foods from our diets could result in health problems associated with consuming an unbalanced diet. In addition, under cooking of food represents a much more definable hazard than ACR from foodborne pathogens which affect millions of people each year resulting in thousands of deaths. Also, more than 200 research projects have been initiated to better understand the risks to humans of dietary exposure to ACR (EFSA 2005; FAO/WHO 2005). Another WHO/FAO meeting is scheduled in early 2005 to evaluate some of these results with regard to food safety.

Several reviews of various aspects of ACR residues in food have been published recently. These reviews have covered topics such the chemistry and biochemistry of ACR (Friedman, M., 2003), neurotoxicity (LoPachin, R. M., 2004, Spencer and Schaumburg, 1974), genotoxicity (Besaratnia and Pfeifer, 2004, Dearfield, Abernathy, *et al.*, 1988, IARC 1994;) reproductive toxicity (Dearfield, Abernathy, *et al.*, 1988, Ruden, 2004, Tyl

and Friedman, 2003) and levels of ACR in food products and dietary exposure estimates (Dybing and Sanner, 2003, Konings, Baars, *et al.*, 2003, Svensson, Abramsson, *et al.*, 2003). This review will concentrate mainly on the toxicology of ACR in an attempt to compile the results of the most important studies in this area into one document and provide some discussion of the collective results.

Regulatory Levels

Acrylamide is not a new chemical in our environment. It has been used in various industries for decades (Friedman, M., 2003). It is used as a binding, thickening or flocculating agent in grout, cement, sewage, waste water treatment, pesticide formulations, cosmetics, sugar manufacturing and soil erosion prevention. The polymers of the compound are used in ore processing, food packaging, plastic products and molecular biology laboratories gels for separation of proteins and chromatography (WHO 1985; EU 2002; IARC 1994). Exposure also occurs from cigarette smoke. Permissible levels have been established in drinking water by the World Health Organization at 1 µg/L, EPA at 0.5 µg/L and the European Union at 1 µg/L. Levels in ambient air for occupational settings have been set at exposure levels of 0.3 mg/m³ for 8 (OSHA) or 10 hour (NIOSH) time-weighted averages. Levels for ACR in cosmetics has been set at <0.1 PPM in body care products and <0.5 PPM for other cosmetic products (SCCNFP 1999). Occupational levels of exposure to ACR are estimated to be much greater than exposure levels in the diet (Marsh, Lucas, *et al.*, 1999).

Neurotoxicity

The neurotoxic properties of ACR have been most studied because these are the only toxic effects have been shown both in humans from occupational exposure and from studies in laboratory animals. The understanding of ACR-induced neuropathies is quiet advanced due to more than 30 years research examining the possible mechanisms of action. Numerous excellent reviews of these effects are available so detailed studies will not be presented here (LoPachin, R. M., Balaban, *et al.*, 2003, LoPachin, R. M., Jr. and Lehning, 1994, LoPachin, R. M., Ross, *et al.*, 2002, Spencer and Schaumburg, 1974, 1974, Tilson, 1981). Studies in several species of laboratory animals such as cats, rats, mice, guinea pigs, rabbits and monkeys (Miller, M. S. and Spencer, 1985) have shown that repeated daily exposure at levels of 0.5-50 mg ACR/kg/da result in a triad of effects such as hind limb foot splay, ataxia, and skeletal muscle weakness as measured by decreased fore and hind limb grip strength. The neurotoxic effects of ACR in humans in occupational settings have been documented (LoPachin, R. M., 2004, Spencer and Schaumburg, 1974). As noted above, neurotoxicity was recently observed in construction workers using a water-proofing sealing gel that contained ACR (Hagmar, Tornqvist, *et al.*, 2001). The clinical signs were of peripheral neuropathy which manifested as tingling and numbness of the hands and feet, weak legs and loss of toe reflexes, all of which were reversible (Hagmar, Tornqvist, *et al.*, 2001). Longer exposures resulted in cerebellar dysfunction, excessive tiredness, ataxia and some central neuropathy, which was also reversible in most cases. The mechanism for neurotoxicity by ACR is thought to be due to interference with kinesin-related motor proteins in neurofilaments that are involved in fast antergrade transport of nerve signals between axons (Sickles, Brady, *et al.*, 1996). Inhibition of these motor proteins and trans-axonal transport of nerve growth factors

results in impaired molecular transport from the cell body to the distal axon which can cause a dying back of the nerve body. The neurotoxicity and this mechanism of action also have important implications in the observed genotoxic and reproductive toxicity of ACR seen in animals. These kinesin motor proteins have important functions in cell division and sperm activity (see Reproductive Toxicity and Genotoxicity sections below; (Tyl and Friedman, 2003). The most commonly used No Observable Adverse Effect Level (NOAEL) for neurotoxicity of ACR exposure in animals is 0.5 mg/kg bw/da and the Lowest Observable Adverse Effect Level (LOAEL) is 2 mg/kg bw/da (Johnson, Gorzinski, *et al.*, 1986, Spencer and Schaumburg, 1974ab). These levels are well above the dietary exposure estimates of the World Health Organization (WHO 2002) of 0.001mg/kg bw/da commonly used in risk assessment models and provide about a 500-fold safety margin. The scientific consensus is that exposure of humans to the relatively low levels of ACR in the diet will not result in clinical neuropathy.

Reproductive Toxicity

Reproductive toxicity has also been observed in laboratory animals exposed to high levels of ACR (Dearfield, Abernathy, *et al.*, 1988, Tyl and Friedman, 2003). The NOAEL for reproductive toxicity has been estimated to be 2-5 mg/kg bw/da depending on the endpoint of fertility or embryonic death (Tyla, Friedman, *et al.*, 2000). No reproductive toxicities have been reported in humans. The NOAEL for reproductive effects is at least 4 times higher than that for neurotoxicity (WHO 2002) and 2000 times greater than estimated dietary exposures (Dybing and Sanner, 2003, Konings, Baars, *et al.*, 2003). Therefore, it is highly unlikely that any reproductive toxicity in humans would result from dietary exposure to ACR. Some of the most relevant studies on reproductive effects in animals are summarized below. Mice exposed to ACR in the drinking water at doses of 1.25 to 24 mg/kg/da for 4 weeks had decreased fertility rates and litter sizes, increased resorption rates, abnormal sperm and decreased sperm counts (Sakamoto and Hashimoto, 1986). Male rats exposed to 4.2 to 7.9 mg/kg/da in drinking water for 10 weeks had reduced copulatory and mounting activity, reduced fertility rates, decreased numbers of sperm deposited in the uterus and decreased pup weights (Zenick, Hope, *et al.*, 1986). Mice exposed to 35.5 mg/kg twice weekly orally for 8-10 weeks showed testicular atrophy, decreased weight of testes and degeneration of the epithelial cells of the seminiferous tubules (Hashimoto and Tanii, 1985). Similar effects on testes were seen in rats exposed subchronically to ACR at 20 mg/kg/da (Burek, Albee, *et al.*, 1980). Several multigeneration studies in rodents have also shown effects of ACR exposure. Mice given 3, 10 or 30 PPM ACR in drinking water for 14 weeks in a continuous breeding study had reduced live litter sizes in the F1 generation (Chapin, Fail, *et al.*, 1995). Studies by Tyl *et al.* (Tyl, Marr, *et al.*, 2000, Tyl, Friedman, *et al.*, 2000) in rats given doses of ACR ranging from 0.5 to 60 mg/kg/da in drinking water showed reproductive effects in generations F0 through F2 at the higher doses. These effects included decreased numbers of live pups, survival of pups and reduced mating behavior. Reduced hormone levels, testosterone and prolactin, have also been reported in rats treated with ACR (Ali, Hong, *et al.*, 1983). A number of studies have also shown dominant lethal effects in rats and mice exposed to high levels of ACR in the drinking water (Chapin, Fail, *et al.*, 1995, Shelby, Cain, *et al.*, 1986, Smith, Zenick, *et al.*, 1986, Tyl, Marr, *et al.*, 2000, Tyl, Friedman, *et al.*, 2000, Zenick, Hope, *et al.*, 1986). These have been demonstrated mostly

by increased pre and post implantation losses. It is interesting that most of the above studies indicate that the effects on reproduction are almost exclusively due to effects on males (Chapin, Fail, *et al.*, 1995, Hashimoto, Sakamoto, *et al.*, 1981, Sakamoto and Hashimoto, 1986, Smith, Zenick, *et al.*, 1986, Sublet, Zenick, *et al.*, 1989, Tyl and Friedman, 2003, Wise, Gordon, *et al.*, 1995, Zenick, Hope, *et al.*, 1986). Very little evidence is available to indicate any primary effect directly on the female reproductive system. Some studies have shown maternal toxicity occurs before reproductive toxicity in females (Field, Price, *et al.*, 1990, Friedman, M. A., Tyl, *et al.*, 1999; Field *et al.* 1990; Sleet *et al.* 1998)

The relationship of neurotoxicity and reproductive toxicity has been the subject of several studies with mixed results (Chapin, Fail, *et al.*, 1995, Costa, Deng, *et al.*, 1992, Sakamoto and Hashimoto, 1986, Tyl, Marr, *et al.*, 2000, Tyl, Friedman, *et al.*, 2000). There has been considerable discussion that neurotoxicity may affect male reproduction. One theory is that neurotoxicity affects mating behavior. Several studies have shown that one of the neurotoxic effects of ACR in rats is a weakness of the hind limbs, reduced hind limb grip strength and increased foot splay (Hashimoto, Sakamoto, *et al.*, 1981, Sakamoto and Hashimoto, 1986, Tyl, Friedman, *et al.*, 2000). This reduced hind limb function could impair mounting responses, copulatory behavior and intromission (entry) (Zenick, Hope, *et al.*, 1986). Dysfunctional intromission could also affect the proper deposition of sperm in the vagina and uterus and subsequent hormonal events that lead to stimulation of reproductive hormones and implantation. In addition, erectile function could be reduced due to nerve damage in the penis (Tyl and Friedman, 2003). Another theory is that the mechanism for reproductive toxicity and neurotoxicity are both mediated through effects on the kinesin motor proteins (Tyl, Marr, *et al.*, 2000). These kinesin proteins are found in the flagella of sperm as well as the nervous system and other tissues ((Miller, M. G., Mulholland, *et al.*, 1999). Interference with these proteins could reduce sperm motility and fertilization events (Tyl and Friedman, 2003, Tyl, Marr, *et al.*, 2000, Tyl, Friedman, *et al.*, 2000). The kinesin motor proteins are also involved in cell division (Adler, Zouh, *et al.*, 1993, Shiraishi, 1978, Sickles, Brady, *et al.*, 1996). They are an integral part of the spindle fibers which attach to and pull apart chromosomes during the metaphase of cell division. This could be the mechanism for the clastogenic effects seen in ACR exposure. It could also be the mechanism of effects on germ cells that result in dominant lethal and heritable translocation effects. These could occur without any direct effects on DNA *per se*. Other mechanisms of ACR on reproduction in rodents could be from alkylation of sulfhydryl groups on unique proteins, such as protamine, in the sperm head and tail (Sega, 1991, Sega, Alcota, *et al.*, 1989) This could affect sperm penetration and cause the pre-implantation losses seen in some dominant lethal studies (Dearfield, Douglas, *et al.*, 1995, Tyl, Marr, *et al.*, 2000). A similar mechanism has been proposed for effects of ACR on DNA proteins without direct effects on DNA (see genotoxicity section). One other mechanism by which ACR may exert its effects via protein sulfhydryl groups is by depletion of glutathione. About 50% of ingested ACR is metabolized by the P450 enzyme, CYP2E1, to the metabolite, glycidamide (GLY)(Sumner, MacNeela, *et al.*, 1992). Both the metabolite and the parent ACR are then conjugated to glutathione by glutathione-S-transferase and excreted in the urine. This glutathione system is also responsible for regeneration of sulfhydryl groups for amino acid and proteins. When glutathione is depleted, it is slow to regenerate. Therefore, high levels of ACR could

deplete glutathione levels and reduce protein function via lack of sulfhydryl groups. Lastly, exposure to ACR has also been reported to reduce serum testosterone and prolactin levels (Ali, Hong, *et al.*, 1983). This could result in the testicular atrophy and decreased sperm development and motility, which has been reported following ACR exposure in rodent studies (Burek, Albee, *et al.*, 1980, Hashimoto and Tanii, 1985). Additional research is needed to clarify the relationship of neurotoxicity and reproductive toxicity and the most relevant mechanisms.

Genotoxicity

The genotoxicity of ACR and its major metabolite, GLY have been the subject of several reviews (Dearfield, Abernathy, *et al.*, 1988, Dearfield, Douglas, *et al.*, 1995; IARC 1994). One of the important parameters in assessment of potential carcinogens is their capacity to cause genetic damage. The most significant damage is considered to be a direct action on the DNA molecule which can be measured by specific gene locus or point mutation assays. A number of tests are accepted by regulatory agencies which have been validated to reflect these mutagenic effects of chemicals. These include the *in vitro* prokaryote systems such as the Ames forward and reverse bacterial mutations tests, usually done in strains of *Salmonella* bacteria. The most common tests in eukaryote systems are the thymine kinase (TK) or hypoxanthine-guanine phosphoribosyl transferase (HGPRT) forward mutations of mouse lymphoma or the Chinese hamster ovary (CHO) cell lines. The classical *in vitro* tests in bacterial systems using ACR have been negative (Bull, Robinson, *et al.*, 1984a, Dearfield, Abernathy, *et al.*, 1988, Hashimoto and Tanii, 1985, Knaap, Kramers, *et al.*, 1988, Tsuda, Shimizu, *et al.*, 1993). This chemical does not seem to be a mutagen in prokaryote cells even in the presence of liver microsomal enzyme activators (S9 fractions). The results of testing for point mutations in mammalian cell lines such as the TK forward mutation in mouse lymphocytes or the HGPRT assay in CHO cells or mouse lymphocytes has shown mixed results. Tsuda *et al.* (Tsuda, Shimizu, *et al.*, 1993) failed to show any mutagenic effects of ACR in the HGPRT assay in CHO cells at high doses. Conversely, Knaap *et al.* (Knaap, Kramers, *et al.*, 1988) also reported no effects in bacterial assay but saw ACR activity in the HGPRT assay and TK assay in mouse lymphocytes at high doses (Dearfield, Douglas, *et al.*, 1995, Moore, Amtower, *et al.*, 1987). Moore *et al.* (Moore, Amtower, *et al.*, 1987) showed an increased frequency of mutations in the TK assay after ACR exposure, but attributed this to a clastogenic effect and not due to point mutations based on the characteristics of the colonies that formed.

In contrast, Besaratinia *et al.* (Besaratinia and Pfeifer, 2003) reported mutations were induced in the cII transgene in mouse fibroblasts but only at very high doses. They also found no direct correlation between mutations and DNA adducts since the profile of adducts did not match the mutation sites. In a later study, these investigators reported the high doses of ACR induced mutations in the same cII transgene and in the TP3 gene that codes for the tumor suppressor protein P53 (Besaratinia and Pfeifer, 2004). They determined this effect was related mostly to the epoxy metabolite of ACR, GLY, and this was limited by the metabolism of the parent compound by the P450 microsomal enzyme system. They also questioned whether the high doses which cause these effects are reasonably achievable in the diet. Other studies fail to show a correlation of GLY-induced adducts and the sites where tumors develop (Sergerback *et al.*, 1995). One other

study showed a weak mutagenic activity in the transgenic MutaMouse lacZ gene in mice given ACR (Hoorn, Custer, *et al.*, 1993). Other assays that are commonly used to assess genotoxicity are designed to detect general damage to DNA without reference to specific genes. These assays are designed to measure effects such as clastogenesis, chromosomal breakage or other chromosomal aberrations. They include assays such as sister chromatid exchange, unscheduled DNA synthesis, micronuclei formation, the comet assay, or chromosomal aberration assays such as the cytogenetic bone marrow assays or tests for polyploidy or aneuploidy. Although ACR does not appear to be a strong classical mutagen, it does appear to damage DNA by some direct or indirect mechanism. This activity is suggested by positive results in general *in vitro* DNA damage assays such as unscheduled DNA synthesis (Lafferty, Kamendulis, *et al.*, 2004) and sister chromatid exchange (Knaap, Kramers, *et al.*, 1988, Russo, Gabbani, *et al.*, 1994, Tsuda, Shimizu, *et al.*, 1993). These investigators judged ACR to be a classic clastogen without mutagenic potential. Exposure of animals to ACR has also resulted in chromosomal damage as measured by increased occurrence of cellular micronuclei in either bone marrow polychromatic erythrocytes (Adler, Ingwersen, *et al.*, 1988, Cihak and Vontorkova, 1988, Dobrzynska and Gajewski, 2000, Knaap, Kramers, *et al.*, 1988, Paulsson, Kotova, *et al.*, 2003), sperm cells (Collins, B. W., Howard, *et al.*, 1992, Lahdetie, Suutari, *et al.*, 1994, Russo, Gabbani, *et al.*, 1994) or other cell lines (Jie and Jia, 2001). Several studies considered this effect as weak and only evident at higher doses. Chromosomal aberrations have also been noted in several studies in mouse bone marrow cells and spermatogonia (Adler, Ingwersen, *et al.*, 1988, Cihak and Vontorkova, 1988, 1990, Knaap, Kramers, *et al.*, 1988, Tsuda, Shimizu, *et al.*, 1993, Working, Bentley, *et al.*, 1987). Others report chromosomal breakage following exposure to ACR (Jie and Jia, 2001, Nesterova, Durnev, *et al.*, 1999, Shiraishi, 1978, Tsuda, Shimizu, *et al.*, 1993). Most of these effects are considered to be related to the clastogenic effects of ACR or its metabolite, GLY.

Once the potential for genotoxicity has been demonstrated, the capacity of a chemical to induce heritable damage in germ cell lines is usually investigated. The most common are the *in vivo* exposure studies such as the dominant lethal assay and the heritable translocation assay. Several investigators have shown increased germ cell DNA damage using the heritable translocation tests (Adler, Gonda, *et al.*, 2004, Shelby, Cain, *et al.*, 1987) and dominant lethal tests (Adler, Gonda, *et al.*, 2004, Dobrzynska and Gajewski, 2000, Shelby, Cain, *et al.*, 1986, Smith, Zenick, *et al.*, 1986, Working, Bentley, *et al.*, 1987) in rodents. It has been postulated by several investigators that the clastogenic effects of ACR on germ cells may not be by direct interaction with DNA. These effects may be mediated through interference with the kinesin motor proteins that are involved in spindle fiber formation and chromosomal segregation during cell division or alkylation of protamines in sperm (Adler, Baumgartner, *et al.*, 2000, Adler, Zouh, *et al.*, 1993, Costa, Deng, *et al.*, 1992, Shiraishi, 1978, Sickles, Brady, *et al.*, 1996) (see Reproductive Toxicology section of this review). Alternatively, ACR may alkylate DNA proteins via an affinity for sulfhydryl groups resulting in clastogenic effects (Sega, 1991, Sega, Alcota, *et al.*, 1989). Also, some of the genotoxic effects have been attributed to one of the major metabolites of ACR, GLY. This is a reactive epoxide of ACR formed after biotransformation by the P450 monooxygenase CYP2E1 and has been shown to form

adducts with DNA and proteins (Adler, Baumgartner, *et al.*, 2000, Besaratinia and Pfeifer, 2004, Dearfield, Douglas, *et al.*, 1995, Generoso, Sega, *et al.*, 1996, Paulsson, Kotova, *et al.*, 2003). Even though there are reasonable questions about the mechanisms by which ACR may act, there is convincing evidence that it does affect DNA integrity either by genotoxic or epigenetic mechanisms. This is an important distinction because epigenetic actions are usually more dose related and reversible. They also have thresholds of exposure below which their effects are negligible. These factors have implications in the application risk assessment models where a genotoxic mechanism of action is assumed (see Risk Assessment section below)

Another type of test that is often done to indicate potential carcinogenicity of a chemical is its capacity to induce cellular transformations *in vitro*. Results from these studies are also mixed for ACR. It has been shown to cause cellular transformations in some cell lines *in vitro* but not others (IARC 1994). Park *et al.* (Park, Kamendulis, *et al.*, 2002) reported that ACR exposure induced transformation Syrian of hamster ovary cells. They concluded that this effect was due to interaction of ACR with sulfhydryl groups on proteins and DNA and therefore was acting by epigenetic mechanisms without direct effects on DNA. Others have reported transformation of CH/10T1/2 and NIH/3T3 mouse fibroblast cells (Banerjee and Segal, 1986) or BALB/c3T3 following exposure to ACR (Tsuda, Shimizu, *et al.*, 1993).

Carcinogenicity

Acrylamide is classified as a “probable human carcinogen” (IARC 1994). The basis for this classification is several fold. First, there is insufficient evidence of any carcinogenic effects in humans from epidemiologic studies or occupational exposure. Second, animals exposed to high doses in the drinking water for prolonged periods develop multiple tumors at multiple sites in both sexes. Third, ACR has been shown to be genotoxic in cell culture by *in vitro* tests and *in vivo* animal models. Lastly, ACR has a structure similar to other carcinogens, vinyl carbamate and acrylonitrile.

Several chronic and high intermittent dose studies were considered in the classification of ACR as a probable human carcinogen (IARC 1994). Male and female F344 rats were exposed to ACR in the drinking water at doses of 0.01, 0.1, 0.5 and 2 mg/kg/da for two years (Johnson, Gorzinski, *et al.*, 1986). Female rats given the high dose had increased incidence of tumors of the mammary gland, thyroid gland, oral cavity, uterus, clitoral gland and central nervous system. Male rats on the high dose had increased tumors of the thyroid gland and scrotal mesothelium. No significant increase in tumors was seen in animals exposed to the lower three doses compared to the controls. Peripheral neuropathy was also observed in males and females on the high dose. Critical reviews of this study point out several ambiguities (Frankos 1985). There was an unusually high incidence of tumors of the CNS and oral cavity in male controls compared to historical controls for this rat strain. There was an atypical dose response in the male rats with scrotal mesotheliomas. Finally, a sialodacryoadenitis virus infection of experimental and control rats may have affected the study outcome. Because of some of the perceived inconsistencies of this study, an attempt was made to reproduce the results in a later study (Friedman, M. A., Dulak, *et al.*, 1995). Fisher 344 rats were exposed to levels of 0.1, 0.5

and 2.0 mg/kg (males) or 1.0 and 3.0 mg/kg (females) in the drinking water for 106 weeks. Some results of this later study confirmed outcomes of the study by Johnson *et al.* (Johnson, Gorzinski, *et al.*, 1986) but there were significant differences. The earlier study showed higher mortality in female rats exposed to ACR while the second study showed males were more sensitive. The first study reported increased CNS glial tumors which could not be confirmed in the later study. Rats given ACR in the later study failed to develop a variety of tumors reported in the first study including greater numbers of tumors of the CNS, oral cavity, clitoral gland or uterus. It is pointed out in the later paper that the only malignant tumors seen in this study were of the scrotal mesothelium and this tumor is virtually unknown in humans and is peculiar to the rat. They also point out that this tumor may have hormonal etiology and that aging F344 rats have a high incidence of Leydig cell tumors which causes drastic hormonal imbalances (Turek and Desjardins, 1979). It may also bring to question a genotoxic mechanism for tumor induction since many of the reported tumors are of endocrine origin (e.g. mammary, thyroid, reproductive organs). Several other studies have shown ACR exposure can alter hormones, such as testosterone, prolactin and levels and dopamine receptors in rats (Ali, Hong, *et al.*, 1983; Crump 1999; 2000)

Other studies cited in the IARC (1994) risk assessment documents were by Bull *et al.* (Bull, Robinson, *et al.*, 1984a, Bull, Robinson, *et al.*, 1984b). These investigators looked at the effects of ACR in classical tumor initiation/promotion assays in mice. Young female SENCAR mice were exposed to 12.5, 25, or 50 mg/kg ACR either by oral, ip or dermal application six times over a two week period. They were then promoted with 12-O-tetradecanoylphorbol-13-acetate (TPA) for 52 weeks. Mice given ACR and TPA developed more skin tumors and with decreased latency in a dose response manner by all routes of exposure tested. The rats did not develop tumors in the absence of TPA treatment, indicating that ACR was acting as an initiator, but was unable to induce cancer when given alone. Interestingly, *Salmonella*-based tests for point mutations in these studies were negative. The authors postulated that ACR may be acting as a clastogen as a mechanism of initiation rather than the classical mechanism of gene locus or point mutations. In the same study, A/J strain male and female mice were treated with oral doses of 6.25, 12.5, or 25 or injected ip with 1, 3, 10, 30 or 60 mg ACR/kg bw 3 times week for 8 weeks. Lung adenomas were significantly increased in a dose response manner in both male and female mice treated with ACR by either route of exposure. Mice given the 60 mg/kg ip dose of ACR developed frank peripheral neuropathy after several injections and were removed from the study. In a subsequent study (Bull, Robinson, *et al.*, 1984), ICR-Swiss mice were treated with 12.5 to 50 mg/kg ACR three times a week and then promoted with TPA. The mice developed more carcinomas and adenomas of the skin and lung than the control group. It should be noted in these studies that, again, exposure to ACR alone did not induce skin papillomas or squamous cell carcinomas. This occurred only after repeated promotion with TPA, which alone had carcinogenic activity. These mice commonly develop lung tumors and exposure to ACR seems more to enhance this effect in some manner that may not be related to direct carcinogenicity. A later study by Robinson *et al.* (Robinson, Bull, *et al.*, 1986) reproduced the skin tumor effects seen in the earlier study (Bull, Robinson, *et al.*, 1984) but could not reproduce the lung tumors. Even the skin tumor induction was weak as noted by the authors. It seems

important to note in all these studies that ACR alone was not carcinogenic in the skin tumor model and only caused the lung tumors at high doses. The fact that ACR may act as an initiator in these models is speculative and may or may not act through genotoxic mechanisms.

Epidemiology Studies

Although there is clear evidence for a carcinogenic effect of ACR when given to laboratory rodents at high doses, this effect in humans exposed to this compound in the diet has not been established. Several epidemiologic studies have failed to show any association of ingestion of ACR in the diet and increases in any kind of cancer. The initial epidemiologic study was very limited in scope. Sorbel *et al.* (Sobel, Bond, *et al.*, 1986) looked at mortality in 371 workers in plants making ACR monomers and polymers with emphasis on cancers at sites observed in animal studies. No relationship to ACR exposure and cancer was observed. The study was inadequate to draw any strong conclusions, however, due to the small population size and, in one case, co-exposure to other potential carcinogenic organic dyes, lack of follow up studies and short-time exposure of some study participants. A larger study was performed by Collins *et al.* (Collins, J. J., Swaen, *et al.*, 1989) looking at risk of cancer in 8500 workers in three plants making water soluble polymers of ACR. This was a 60 year cohort study. No relationship to any kind of increased cancer risk was found. A follow up study of this cohort was done by Marsh *et al.* (Marsh, Lucas, *et al.*, 1999) looking at cancer deaths for the next 11 years from when the first study ended. They found no evidence of an association of ACR exposure and cancer deaths. They did report a continued increase in respiratory cancer seen in the previous study, but this was in a subpopulation of workers in one plant also exposed to muriatic acid. They also found an increase in pancreatic cancer in workers exposed to levels of ACR above 0.3 mg/m³/yr. However, no consistent exposure-response relationships were present to relate to ACR exposure over time. The authors did not consider the pancreatic cancer an ACR-induced effect. There were several weaknesses in this study such as inclusion of short-term workers with limited exposure and incomplete data on smoking history. Some of the non-significant effects seen in respiratory and pancreatic cancer could be caused by smoking. Smoking data was obtained for only about 35% of the exposed group, but about 75% were smokers. One important aspect of this study is magnitude of exposure. The exposure was estimated to be 0.001 mg/m³ or 0.25 mg/m³/yr which is equal to 912.5 mg using an intake of 10m³ and 100% absorption. Daily dietary exposure is estimated to be 0.033 mg/day which is equal to 843 mg for a 70 yr life span. Therefore, the workers in this study were exposed by inhalation every year to over 100% of the average estimated lifetime dietary exposure with no evidence increased cancer risk.

Since the discovery of ACR levels in some foods, several additional epidemiologic studies have been done by Mucci *et al.* (Mucci, Dickman, *et al.*, 2003). The first was a population-based case control study in Sweden. This group examined the incidence of large bowel, kidney and bladder cancer as related to ACR exposure in 14 different foods. These potential sites for cancer were thought to be most relevant due to intestinal exposure to ACR in food and its excretion in the urine. The ACR levels in the food were considered high 300-1200µg/kg or moderate, 30-299 µg/kg. They found no association

between ACR exposure and increased cancer risk. In fact, they saw a reduction in bowel cancer thought to be due to the high fiber in the foods measured. Due to the relatively small size of the study (large bowel cancer 591; bladder cancer 263; kidney cancer 131 and 538 controls) there was limited statistical power to detect small increases in cancer. Subsequently, a larger study was done that concentrated on only renal cancer (Mucci, Lindblad, *et al.*, 2004). Again, there was no association between renal cancer and ACR intake. This Swedish group has done two other studies which are yet unpublished and the data are preliminary. The first examined the relationship of ACR in diet to colon and rectum cancer incidence in 60,000 women over a 12 year period. The second study looked at 49,000 women and breast cancer incidence. No correlations were found in either study that indicates an association with dietary ACR, however, no strong conclusions have been made because of the preliminary nature of the data. The estimated daily intake of ACR in those studies was 31 $\mu\text{g}/\text{da}$, later updated to about 40 $\mu\text{g}/\text{da}$ when coffee was included. In a more specific study design, a large case control study of cancer patients from 1991-2000 was conducted in Italy and Switzerland to examine the relationship between cancer and consumption of fried and baked potatoes (Pelucchi, Franceschi, *et al.*, 2003). They found no increased cancer risk in the oral cavity, pharynx, esophagus, larynx, large bowel, colon, rectum, breast or ovaries that could be associated with ACR in fried or baked potatoes. In fact, they also found a decrease in cancer of the large bowel as reported previously by others.

Risk Assessment

Since there is no epidemiologic evidence that dietary ACR increases the risk of cancer in humans, some regulatory agencies have resorted to the use of risk assessment models to calculate hypothetical risks. Other countries (e.g. UK Independent Committee on Carcinogenicity in Food, Consumer Products and the Environment) will not use these models because, even though the results are highly hypothetical and numbers generated differ greatly between models, they give a false credibility to the process and a perception of reality based on incomplete data. No consensus could be reached at the Food Safety Consultations Meeting by WHO as to how risk assessment models should be used to estimate cancer risk to humans (WHO 2002). Regardless, cancer risk assessment studies have been conducted by regulatory agencies in the United States, Sweden, Norway, the Netherlands, Soviet Union, Europe and international groups such as the World Health Organization and the International Agency for Research on Cancer. Most of these studies have used an average exposure level of 1 μg ACR/kg bw/ da in a 70 kg person as the standard. The study by the Norwegian group estimated an increased cancer incidence of 6/10,000 individuals on average with children and youngsters a little higher based on eating habits (Dybing and Sanner, 2003). Other estimates using this level of exposure have estimated increased incidences of cancer in groups of 10,000 to range from 7 (WHO 1996) to 45 (EPA 1993). The 1 $\mu\text{g}/\text{kg}$ bw/da is considered a high dose based on actually studies that have estimated average daily consumption of ACR in the diet. The estimated average daily intake of ACR in $\mu\text{g}/\text{kg}$ bw/da from several studies has been 0.46-0.49 (Dybing and Sanner, 2003), 0.46 (Konings, Baars, *et al.*, 2003), 0.5 ((Svensson, Abramsson, *et al.*, 2003), 0.3-0.8 (Mucci, Dickman, *et al.*, 2003; FAO/WHO 2003). The exposure estimates also vary with age groups with the highest exposure expected in children based on weight differences. When the actual dietary exposure to ACR is used in

risk estimates, the hypothetical risk of increased cancer incidence is much lower ranging from less than one to 4.5 per 10,000 individuals. A basic problem also exists when estimating dietary exposure levels by these models. Not all foods have been tested for ACR levels and the concentrations vary greatly in foods that have been tested, even within the same food types, brands and batches (Friedman, M., 2003; FAO/WHO 2003). Also, foods with low levels of ACR could account for significant exposure based on volume consumed (e.g. coffee). Conversely, those foods with higher levels may contribute very little.

Fourteen risk assessment studies have recently been reviewed by Ruden (Ruden, 2004). Three of the studies concluded that ACR is not a carcinogen in either animals or humans. These studies have limited basic credibility based on the information used in the model. For example, a study from Russia only used data generated by Russian scientists. Eleven of the studies concluded that ACR is a carcinogen in animals and is likely a carcinogen in humans. They also agree that there is limited data with which to draw conclusions and that the only definitive studies that show the carcinogenicity of ACR are in animals. There has been absolutely no evidence that ACR exposure in the diet is associated with any increased risk of cancer in humans. All epidemiologic studies have been negative (Ruden, 2004 for comprehensive review). However, it has been pointed out by several risk assessors that the epidemiologic studies to date may have lacked the statistical power to detect small increases in cancer rates that may be attributable to ACR exposure in the diet (Hagmar and Tornqvist, 2003; EU 2002;). This is made even more difficult when looking at cancers with a high background incidence or that have common causes. It is estimated that about one-third of the cancers in humans are related to diet, which is a high background. Most of these cancers, however, are not necessarily due to chemicals but many relate malnutrition, mineral deficiencies, fat intake, low fiber, etc., and fewer to natural and environmental chemicals and even less to synthetic chemicals. The estimates on the number of subjects that may be needed to detect increased cancer risks for ACR in food depend on the study assumptions. For example, if 2% of population is exposed to high levels and the relative risk is between 1.015 and 1.05, the number people on the study would need to be 470,000 exposed and 235,000 controls. If the high exposure was increased to 20% of population (1.10), then 15,890 patients would be needed with 7946 controls (Ruden, 2004). This is much larger than any current studies, except for those in progress by the Swedish group which are not yet published (Coughlin 2004).

Other problems associated with risk assessment models which preclude their use by some countries and agencies are the assumptions that must be made to make the models work. The first of these is the assumption that effects seen in animals can be extrapolated to humans. Since often no, or limited, data exist in humans, some consider this extrapolation the only approach. This assumption has proven useful in useful in many instances for non-cancer endpoints but there are notable exceptions, especially when extrapolating carcinogenic effects (Mitka, 2002). For example, the artificial sweetener saccharin induces bladder cancer in rats at high doses but not humans (Cohen, 1999). Heterocyclic amines, formed naturally in cooked meats are carcinogenic in animal studies but there is no evidence of these increasing cancer risk in humans (Augustsson, Skog, *et al.*, 1999). A number of these rodent carcinogens that form naturally from cooking, such as ACR,

polycyclic aromatic hydrocarbons and heterocyclic amines cannot always be readily controlled. Arsenic is a carcinogen in humans but this cannot be reproduced in animal models (Casarett and Doull 2001). There can be inherent differences in sensitivity to carcinogens between humans and rodents based on qualitative or quantitative differences in physiological and metabolic factors that limit extrapolation. Differences between species in the metabolism of ACR have already been reported (Twaddle, McDaniel, *et al.*, 2004). The bioavailability of ACR in humans is virtually unknown, yet risk assessment models assume a 100% uptake of the chemical and that it is the same for the rodent.

A second assumption is that effects seen at high doses in animals can be extrapolated to the effects of low doses in humans. High dose studies are commonly done to increase the incidence or chance of seeing the effects of the chemical in a limited number of animals. However, high doses of the chemical in animals may saturate metabolic pathways or affect the distribution of the chemical in the body causing effects that would not be seen at lower doses. The number of toxic metabolites may be increased with higher doses. Higher doses of the chemical may induce cellular death and cause increased cell division which could increase chances of mutations in dividing cells. This could be a particular problem with ACR. It is metabolized by the Phase I P450 enzyme CYP2E1 into a genotoxic metabolite, GLY (Sumner, MacNeela, *et al.*, 1992). This metabolite is then conjugated by the Phase II enzyme glutathione transferase to glutathione and excreted. The GSH system is very saturable with regard to depletion of glutathione. Once the glutathione levels are depleted some of the original chemical or its metabolites can no longer be excreted and toxicity increases. This could be the case with high doses of ACR and production of the genotoxic metabolite, GLY. This has been shown to be a problem with overdoses of certain pain relievers such as acetaminophen (Mitchell, Jollow, *et al.*, 1973). Glutathione becomes depleted and is not readily regenerated. Also, it has been shown that DNA repair systems are more error-prone as DNA damage is increased. Less damage at lower doses is more readily and accurately repaired (Ehling, Auerbeck, *et al.*, 1983).

A third assumption of risk assessment models to predict cancer risk is the assumption that the chemical is genotoxic and the effects on DNA are the mechanism of cancer induction. As reviewed above, the results of genotoxicity studies with ACR have varied considerably. Although ACR *per se* does appear to cause some genetic damage, it does not seem to be a consistent classic mutagen with strong activity to induce point or gene locus mutations. One of the metabolites, GLY, is a mutagen. Acrylamide appears to act more as a clastogen for which the mechanism of direct action on DNA is unclear. In fact, as noted above, the clastogenic effects of ACR could be due to effects on kinesin proteins which are involved in formation of spindle fibers and separation of chromosomes during the metaphase of cell division, with no direct effect on DNA. Acrylamide or its metabolites may also act by alkylation of proteins associated with DNA, with no direct effects on the DNA. Also, many of the tumors produced by ACR in rodents are of endocrine origin or hormonal-related (thyroid, mammary, reproductive organs, pancreas, etc.). Effects on hormone and endocrine systems can be important in epigenetic induction and promotion of cancer by stimulation of cell division and expansion of background tumors. There is good evidence that genotoxicity may not be the only mechanism

operating in the induction of tumors in animal studies. If epigenetic mechanisms are more important for this chemical, then the risk assessment models are more inappropriate because these indirect carcinogens are much more subject to thresholds and prolonged exposure conditions.

Lastly, it is inherent in risk assessment models for cancer risk that extrapolations be made beyond the data. In most models, a linear extrapolation is used which assumes that there is a direct dose response relationship from effects seen at high doses to what will happen at low doses. This assumption must be made because cancer effects may occur at a low enough frequency that these will not be evident at low doses in small population. Linear dose response effects are not operative for many chemicals at low doses for some of the reasons stated above relating to metabolism, distribution, DNA repair and dose-related mechanisms of action. In fact, some conclude there does not seem to be a linear relationship between dose and carcinogenic effects of ACR in animal studies (Bolt, 2003)

Conclusions

It is clear that ACR is neurotoxic in animals and humans. The neurotoxic effects, however, seem to be only a problem in humans with high level exposure. The lower levels of exposure estimated from dietary sources do not represent a hazard for neurotoxicity in humans.

Acrylamide has reproductive toxicity as demonstrated in animal studies. These effects have not been seen in humans. The mechanism of reproductive effects may or may not be related to the neurotoxic effects. There are data that indicate these effects may be caused by the neurotoxicity and resultant behavioral changes. Alternatively, the effects may be by the same mechanisms as neurotoxicity but through effects on the kinesin motor proteins in reproductive cells. The mechanism may also be by direct interaction with proteins essential to the function of germ cells. Exposure of humans to dietary levels of ACR is not expected to induce any reproductive toxicity.

Acrylamide is a rodent carcinogen when given at high doses or promoted with strong promoting agents. There is no evidence from occupational or dietary exposures that ACR increases cancer risk in humans. All epidemiologic studies are negative although some of these studies may lack the statistical power to detect small increases in cancer incidence related to diet. The mechanism of carcinogenicity in rodents is unclear. Exposure to the chemical causes genetic damage but this may be through indirect effects on proteins involved in cell division or chromosome structure and function and not directly on DNA per se. High incidence of hormonal or endocrine tumors may also suggest epigenetic mechanisms involving hormonal imbalance and increased cell division. It seems likely though that a direct effect on DNA is also a factor, especially from the reactive metabolite, GLY.

There is consensus among regulatory groups in a number of countries that not enough information is available concerning the amount of ACR in different foods. Also, the amount that is there varies greatly even within the same brands and batches. There is also not enough information about the health effects of these low levels of ACR in the diet.

Consequently, no credible food safety group or government agency is recommending any changes in our food choices at this time to avoid foods that contain ACR. This could in fact result in dietary imbalances, nutritional issues or other food safety issues such as under cooked foods.

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